Role of IL-33 Signalling in COVID-19 Inflammatory Status

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During acute infection, the increased levels of pro-inflammatory cytokines, which are involved in the pathology of disease and the development of SARS-CoV-2-induced acute respiratory disease syndrome, the life-threatening form of this infection, are correlated with patient survival and disease severity. IL-33, a key cytokine involved in both innate and adaptive immune responses in mucosal organs, can increase airway inflammation, mucus secretion and Th2 cytokine synthesis in the lungs, following respiratory infections. Similar to cases of exposure to known respiratory virus infections, exposure to SARS-CoV-2 induces the expression of IL-33, correlating with T-cell activation and lung disease severity.

IL-33   COVID-19   cytokine storm   inflammation

1. Introduction

The involvement of various molecules, cytokines and immune cells has been described in COVID-19 (**Figure 1**), but a key concept to highlight is that the cells damaged by SARS-CoV-2 release alarmins, or damage-associated molecular patterns (DAMPs), which act as danger molecules in promoting inflammatory response [1][2]. IL-33, as reported above, is recognized as an alarmin, an expression of cellular damage or infection, whose increased levels in epithelial and endothelial cells recall its pro-inflammatory role in respiratory diseases. In particular, the mature bioactive form of IL-33 requires cleavage by proteases. In the literature, it has been reported that serum IL-33 levels are up-regulated in elderly patients affected by COVID-19, an expression of the epithelial damage induced by the interaction between the airway epithelium and activated immune cells, linking them to severe outcomes [3]. These data imply a key role for IL-33 in COVID-19 pathogenesis. However, SARS-CoV-2-derived papain-like protease (PLpro), an inducer of IL-33 in epithelial cells, could also trigger epithelium-derived IL-33, inducing inflammation in the lungs.

Figure 1. The effects of SARS-CoV-2 on the alveolar epithelium.

It is equally important to highlight that IL-33 can stimulate antiviral cytotoxic T-cell action and the production of antibodies. Stanczak MA. et al., further highlighting the role of IL-33 in COVID-19 immunobiology, reported that the persistent production of IL-33 in response to T-cell activation may be useful in the case of later contact with the virus [4].

Liang et al. infected two human epithelial cell lines, Fadu and LS513, with SARS-CoV-2 in vitro, reporting a significant increase in IL-33 transcript levels in both cell lines at 72 h post-infection [3].

Marcovic SS. et al. analysed the correlation of IL-33 and other innate immunity cytokines with COVID-19 severity in patients with COVID-19 that were divided into two groups (mild/moderate and severe/critical). The authors reported that in a more progressive stage of the disease, increased IL-33 levels are a determinant in lung inflammation favouring the production of innate pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, IL-12 and IL-23 in several target cells, causing the most severe forms of COVID-19. The authors also reported a positive correlation between IL-33 and clinical parameters of COVID-19, in particular higher values of the neutrophil count, C-reactive protein (CRP), D dimer, procalcitonin (PCT), lactate dehydrogenase (LDH), urea, creatinine, creatine kinase CK, ferritin, total bilirubin (TBIL) and aspartate aminotransferase (AST), as well as lower values of lymphocyte and monocyte count and albumin. Serum levels > 332.08 pg/mL of IL-33 were considered a factor related to the risk of increased severity of COVID-19 [5]. The same results were reported by Wang et al. and by Rubio-Sánchez et al. [6][7].

Meidaninikjeh S. et al. and Miyazawa M. reported that cellular compositions of lung infiltrates in patients with COVID-19 pneumonia are different depending on the progression of the disease. In detail, in patients with moderate pneumonia, infiltrates are made up mainly of lymphoid and dendritic cells, while in patients with the severe form of the disease there is a massive infiltration of macrophages and neutrophils [8][9]. IL-33 induces neutrophil migration via macrophage-derived CXCL1 and CXCL2, whereas neutrophil elastase and cathepsin G

are responsible for IL-33 processing and maturation, which leads to inflammatory responses. In agreement with these data, Marcovic SS. et al. highlighted an increased neutrophil count in patients with severe COVID-19 [5].

Focusing on seroconversion, in the literature, it has been reported that IL-33 clustered together with anti-receptor binding domain (RBD) IgG in moderate and severe cases [10]. Stanczak M.A. et al. reported that the SARS-CoV-2-infection-induced production of IL-33 clustered together with IgG titres and that after recovery from COVID-19 patients still had persistent circulating PBMCs that produced IL-33 in response to virus-specific T-cell activation, correlating with seropositivity [4]. Zeng G. et al. studied soluble ST2 (sST2, a cardiac biomarker) levels among COVID-19 patients and a possible relationship between inflammatory status and disease severity, highlighting a positive correlation between serum sST2 levels and CRP, and a negative correlation between CD4+ and CD8+ T lymphocyte counts and sST2. In particular, Zeng Z. et al. analysed the relationship of serum sST2 to lymphocyte subsets, reporting that CD3+CD4+ and CD3+CD8+ lymphocyte absolute counts were negatively correlated with the levels of serum sST2 in patients with COVID-19, highlighting that the elevated serum sST2 may favour the dysfunction of T cells in COVID-19 progression [11]. This concurs with many studies reporting that during COVID-19 infection patients are often characterized by low lymphocyte counts and impaired cytotoxic activity [12]. Gaurav et al. compared lung sections of COVID-19 patients to those of normal patients and patients with chronic inflammatory lung diseases, such as IPF and COPD, highlighting that tissue IL-33 levels were higher among the latter. Indeed, patients with COVID-19 had very low IL-33 expression, which was significantly reduced compared to that of control subjects. IL-33 levels were increased in post-COVID fibrosis and were higher compared to the levels in patients affected by COPD and IPF [13].

2. Cytokine Storm, IL-33 Effects and Thrombosis in COVID 19 Infection

The most frequent complication of systemic infections is the triggering of the coagulation process, which can exhibit a wide variety of clinical symptoms fluctuating from subclinical expression, represented by increased laboratory indicators for fibrin and thrombin products, to the appearance of thrombosis and disseminated intravascular coagulation [14].

Regarding COVID-19 patients, the devastating occurrence of uncontrolled coagulopathy is a powerful prognosticator of mortality in infected subjects [15]. However, the genesis of hypercoagulable condition and thrombosis correlated to COVID-19 is uncertain [16][17][18]. COVID-19 infection likely provokes a condition of immune system hyperstimulation, which has been defined as immunothrombosis (**Figure 2**), in which stimulated neutrophils react with activated platelets and the coagulation factors, causing intravascular clot generation in all vessels. It is supposed that the overstated immunothrombosis that occurs within pulmonary microvessels is the leading cause of COVID-19 symptoms [19][20].
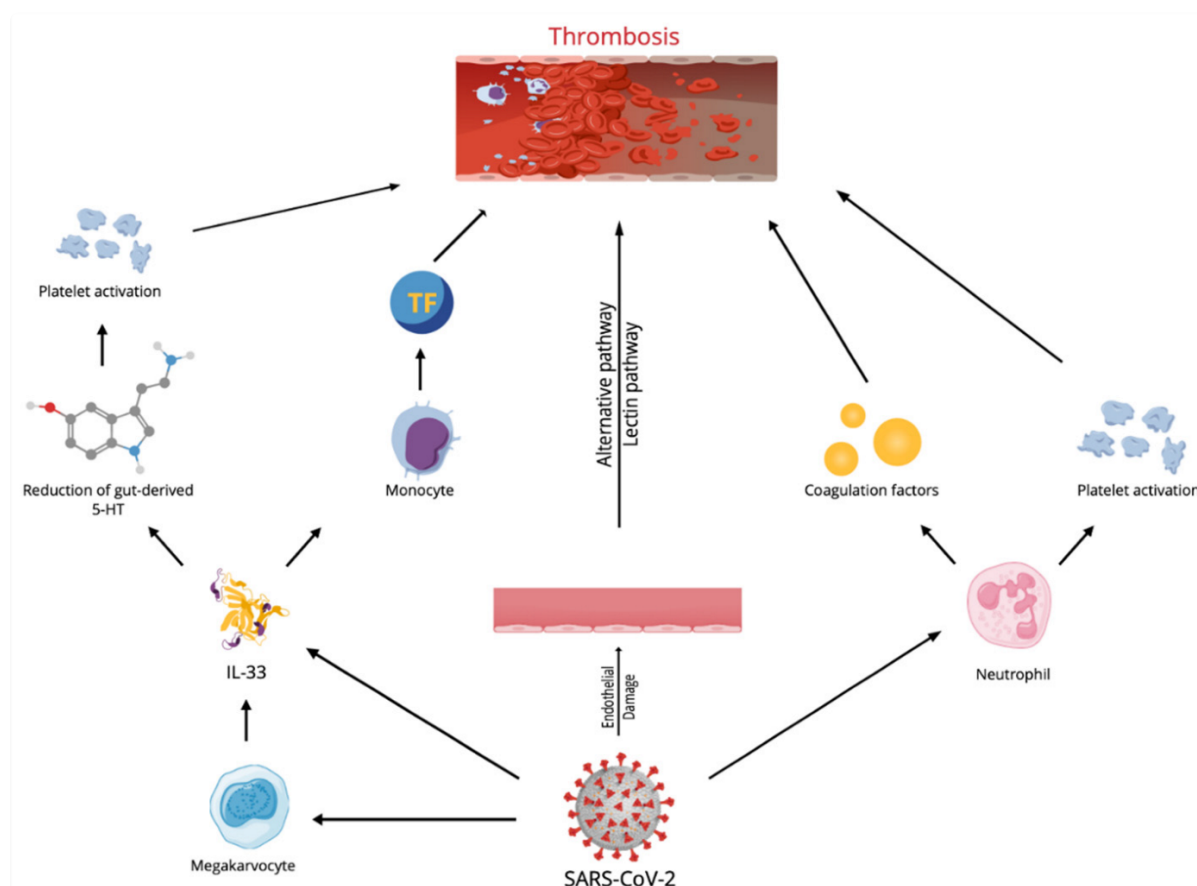
Figure 2. Mechanisms responsible for immunothrombosis in SARS-CoV-2 infection. TF = tissue factor.

A different motive for stimulating the coagulation cascade is represented by endothelial cell damage and activation by SARS-CoV-2 infection itself. Assessment of skin and pulmonary autopsies of subjects who died of COVID-19 infection demonstrated the presence of thrombosis and microvascular damage, coherent with severe, widespread stimulation of alternative and lectin-founded paths of the complement system [21] and successive stimulation of the clotting cascade, provoking fibrin accumulation [22]. The resulting hypercoagulable condition is the main reason for venous thromboembolism, pulmonary embolism and deep venous thrombosis of the lower extremities, which has been reported in subjects affected by COVID-19.

Recently, an in vitro experiment confirmed the role of the cytokine storm in the generation of thrombosis in COVID-19 patients [23], and IL-33 plays an essential role in the cytokine storm, especially regarding the triggering of pro-thrombotic phenomena.

Indeed, IL-33 stimulates endothelial cells (ECs), causing the onset of an inflammatory phenotype via an increase in different molecules such as intercellular adhesion molecule-1, vascular cell adhesion molecule-1, monocyte chemoattractant protein-1 and endothelial-selectin or cytokines such as IL-6 and Il-8 [24][25]. IL-33 also supports angiogenesis and regulates the proteolytic ability of ECs by easing the generation of plasminogen activator inhibitor type-1 and urokinase-type plasminogen activator [26][27]. In a different patient setting, several reports showed that IL-33 serum concentrations are correlated with reduced survival in subjects with ST elevation

myocardial infarction [28][29], while an experiment demonstrated that increased levels of IL-33 after coronary stent implantation are correlated with coronary restenosis [30].

Furthermore, tissue factor (TF) is the main factor of blood coagulation and has a critical effect in the onset of thrombotic events. IL-33 stimulated TF mRNA and protein in umbilical and coronary artery ECs in an ST2- and NF-κB-dependent modality, but this increase was IL-1-independent. IL-33-exposed ECs decreased the coagulation time of plasma and whole blood, while in atherosclerotic plaques, TF mRNA was related to IL-33 mRNA generation. Moreover, IL-33 and TF protein were found in the same location of clot generation in plaques of subjects with carotid stenosis. Thus, IL-33 could increase thrombotic ability and operate on thrombus formation via the production of TF in ECs [31].

However, the effects of IL-33 on TF production could also occur via other mechanisms. Monocytes and monocyte-derived microvesicles (MVs) are one of the principal origins of TF. Increased monocyte-derived TF and an increased circulating amount of procoagulant MVs participate in the establishment of a prothrombotic state. IL-33 was reported to induce a time- and dose-dependent increase in monocyte TF concentrations. IL-33-exposed monocytes also released CD14+TF+ MVs and IL-33 was reported to increase the TF action of monocytes and monocyte-originated MVs. Intermediate monocytes (IMs) displayed the greatest receptor expression, with a reduced expression on non-classical monocytes (NCMs) and classical monocytes (CMs). IL-33 caused a relevant increase in TF only in the IM, while a reduced expression was reported in NCMs, and no increase in CMs was noted. Thus, IL-33 may further participate in the establishment of a prothrombotic condition [32].

Beside the potent effect on TF generation, in vitro experiments demonstrated IL-33 was able to reduce the production of TF pathway inhibitor in ECs. This effect influences the coagulation time of whole blood and plasma ex vivo [33]. Furthermore, IL-33-caused generation of adhesion molecules on ECs could cause an increased binding and stimulation of white cells and leukocyte-originated MVs to ECs [34][35][36].

Furthermore, the strict, bidirectional relationships between IL-33 and platelets and their precursors are of particular significance. A study performed on an animal experimental model of intestinal inflammation displayed the precise effect of IL-33 in stimulating platelets. Inflammation increases systemic platelet triggering and blood coagulation, generating increased concentrations of IL-33 and provoking platelet activation through a rise in 5-HT release. More relevantly, authors assessed that deficiency of epithelial-originated IL-33 reduced 5-HT concentration, causing an altered platelet activation [37].

Finally, though platelets are the most relevant effectors of thrombosis, they are also immune cells. Platelets interact with immune cells, modifying their activity, maturation and proliferation, interfering with all expression of the immune response. Megakaryocytes (Mks) are the bone marrow precursors of circulating platelets, and until lately Mks were only believed to be bone-marrow-occupant cells. However, Mks also are present in the lung, and these Mks present higher numbers of immune molecules than bone marrow Mks. A study attempted to assess the immune activities of lung Mks (MkLs). They reported that MkLs had gene expression profiles that are analogous to antigen-presenting cells, and the immune phenotype was variable and regulated by the tissue immune milieu, as

demonstrated by bone marrow Mks presenting an MkL-like phenotype after exposure to immune molecules, such as IL-33. In vitro and in vivo experiments showed that MkL assumed and managed antigenic proteins and pathogens, stimulated CD4+T cell triggering in an MHC II-dependent modality [38] and may have a central role in IL-33-induced thrombosis in COVID 19 patients.

A direct or indirect intervention aimed at modulating the effects of the IL-33 on the homeostasis of the coagulative system could guarantee an effective prophylactic or therapeutic action in the catastrophic thrombosis induced by the cytokine storm that complicates the course of COVID-19 infection.

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